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SAFETY ASSESSMENT OF SUGAR DUSTING TREATMENTS BY ANALYSIS OF HYGIENIC BEHAVIOR IN HONEY BEE COLONIES

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Abstract - The hygienic behavior in honey bees is a dominant natural defense mechanism against brood diseases. In this study, the influence of sugar dusting treatments on hygienic behavior was evaluated in 44 strong honey bee colonies. Three doses of pulverized sugar, 20, 30 and 40 g, each applied at three-, seven- and fourteen-day intervals were tested. The percentage of cleaned cells (PCC) in the total number of those with pin-killed brood served as a measure of the hygienic potential. The effect was dependent on the frequency of treatments: all doses applied every third and seventh day significantly ($p<0.001$) decreased the PCC in comparison with the untreated control colonies. Nevertheless, sugar did not threaten the hygienic potential, as PPC values remained above 94% following all treatments. Thus, it can be concluded that the tested sugar treatments are safe and can be justifiably implemented into integrated pest management strategies to control *Varroa destructor*.

Key words: Apis mellifera, hygienic behavior, safe treatments, sugar dusting, *Varroa destructor*

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INTRODUCTION

Not unlike other social insects, honey bees (*Apis mellifera* L.) possess both individual and social immunity (reviewed in Evans and Spivak, 2010). Individual bees enlist mechanical, physiological, and immunological defenses against pathogens (Wilson-Rich et al., 2009). Although these serve to minimize the threat to the whole colony, immune responses in an individual are enhanced when coordinated interactions among nestmates result in colony-level immune responses. The collective defense against parasites, arising from the behavioral cooperation among individuals, is termed "social immunity" (Cremer et al., 2007; Evans and Spivak, 2010).

Among a variety of individual and colony-level defenses evolved in the honeybee, hygienic behavior deserves special attention. It is a classical example of social defense, whereby workers identify and remove a diseased and parasitized brood (reviewed in Boecking and Spivak, 1999; Evans and Spivak, 2010).

Hygienic behavior in honey bees is the dominant defense mechanism against brood diseases, such as American foulbrood and chalkbrood, but also against *Varroa destructor* mites infesting brood cells (reviewed in Boecking and Spivak, 1999; Wilson-Rich et al., 2009; Evans and Spivak, 2010). The knowledge of this mechanism is a prerequisite for the safe control of bee pathogens, including *V. destructor*, the greatest threat to apiculture worldwide. Safe control methods imply

the avoidance of drugs and other chemicals, given their numerous side effects on the honey bee (Gregorc and Bowen, 2000; Loucif-Ayad et al., 2008; Gregorc and Ellis, 2011), bee products (Bogdanov, 2006; Martel et al., 2007; Lodesani, 2008) and consumers (Stanimirovic et al., 2005a, 2007, 2010; Stevanovic et al., 2006, 2008). Moreover, synthetic acaricides may lead to resistance in *Varroa* mites (Milani, 1999; Milani and Della Vedova, 2002), the necessity for higher doses and more frequent application, thus, resulting in the increase in side effects. In addition, they result in the disturbance of natural mechanisms in parasites which control their behavior and limit the damage they do to the host to amounts which are considered safe, given that the survival of hosts is a prerequisite for that of the parasite. However, no chemical treatment of a honeybee disease, even if successful at the colony level in the short-term, has eradicated the pathogen at the population level, particularly if it has a high transmission rate and infectivity. Thus, it is necessary to develop safe methods for the control of honey bee pathogens, including parasites, which may enable the improvement of complementary strategies for disease control and secure the quality and safety of honey and other bee products (Moritz et al., 2010).

Treatment of bee colonies with safe substances and the stimulation of their defense mechanisms, for example grooming behavior which is considered important against *Varroa* mites (reviewed in Evans and Spivak, 2010), are considered safe means of combat against ectoparasites. Pulverized sucrose may be used, which has been proven to be non-toxic (Pettis et al. 2004) and is applicable throughout the year, including the period of honey harvest; it is capable of stimulating egg laying, brood nursing and productivity of the colony, and what is possibly most important, it does not lead to resistance (Cirkovic, 2011). Its additional positive effect is the stimulation of grooming behavior in honey bees (Stevanovic, 2007). Besides this, it produces no adverse effects on brood development, adult bee population, queen and colony strength (Fakhimzadeh, 2001; Ellis et al., 2009; Cirkovic, 2011; Stanimirovic et al., 2011). For these reasons and the positive effects in knocking-down mites and lessening the ectoparasite burden in the hives (Fakhimzadeh, 2000, 2001; Stanimirovic et al., 2011), sugar dusting has been proposed as an ecological method which should be implemented in integrated pest management strategies on the control of *V. destructor* (Stanimirovic et al., 2011). However, it is unknown how these sugar treatments influence hygienic behavior, which was the goal of this research.

MATERIALS AND METHODS

The research was conducted on 44 strong honey bee colonies at the experimental apiary of the Faculty of Veterinary Medicine, Belgrade University. In order to homogenize the experimental conditions, the colonies were equalized as described by Wantuch and Tarpy (2009), with equal food reserves and kept under the same environmental conditions. The expression of honey bee hygienic potential was evaluated using the pin-killed brood (PKB) assay and criteria as described in Stanimirovic et al. (2008). The percentage of cleaned cells (PCC) recorded was used as a measure of hygienic behavior.

The colonies were randomly divided into 11 groups, each consisting of four hives. Two groups were control: a negative (K-), untreated, and a positive (K+), which was treated with an acaricide amitraz (standard procedure of fumigation). The remaining nine groups were dusted with powdered sugar: three with 20 g, three with 30 g and three with 40 g. Each dose of sugar was applied at three-, seven- and at fourteen-day intervals. Groups treated at three-day intervals received nine treatments, at seven-day intervals five, whilst those treated at 14-day intervals were dusted three times. All groups dusted with sugar were labeled with a three-part symbol: the first, ST, meaning 'sugar treated'; the second describing the quantity of sugar applied (20, 30 or 40 g), and the third, the dynamics of treatment (3, 7 and 14 – three, seven and fourteen-day intervals, respectively). Sugar dust was prepared and applied as explained previously (Stanimirovic et al., 2011). The evaluation of hygienic behavior was performed on three occa-

Table 1. Parameters of descriptive statistics of hygienic potential in experimental groups of hives before and after treatment

Time of assessment	Experimental group	$\overline{X}$	Min	Max	SD	SE	Cv (%)
Before treatment	ST-20-3	98.323	97.970	98.900	0.417	0.209	0.424
	ST-20-7	97.975	96.690	99.170	1.015	0.507	1.036
	ST-20-14	99.055	98.700	99.450	0.326	0.163	0.329
	ST-30-3	97.313	96.970	97.520	0.284	0.132	0.271
	ST-30-7	98.003	96.690	99.450	1.377	0.689	1.405
	ST-30-14	97.038	95.320	99.170	1.594	0.797	1.643
	ST-40-3	97.565	96.420	98.070	0.771	0.386	0.791
	ST-40-7	97.175	96.140	98.070	1.040	0.520	1.070
	ST-40-14	97.728	96.970	98.350	0.611	0.306	0.626
	K+	86.225	84.020	88.150	1.727	0.864	2.003
	K-	97.453	95.870	98.900	1.531	0.765	1.571
After treatment	ST-20-3	94.763	94.210	95.590	0.676	0.338	0.714
	ST-20-7	96.143	95.590	96.420	0.391	0.196	0.407
	ST-20-14	97.105	96.420	98.070	0.728	0.364	0.750
	ST-30-3	94.005	93.390	94.780	0.608	0.304	0.647
	ST-30-7	94.693	94.210	95.040	0.348	0.174	0.367
	ST-30-14	97.313	96.690	98.070	0.611	0.306	0.628
	ST-40-3	95.248	94.760	95.870	0.474	0.237	0.497
	ST-40-7	94.285	92.840	95.870	1.533	0.767	1.626
	ST-40-14	96.900	96.420	97.520	0.470	0.235	0.485
	K+	91.115	90.080	92.290	0.913	0.456	1.002
	K-	98.693	97.800	99.450	0.756	0.378	0.766

sions at 15-day intervals, both before and following the treatments.

Analysis of the results was performed with descriptive and analytical statistics with Statistica 6 software (StatSoft, Inc, USA). The mean ($\overline{X}$) was used as a measure of central tendency. The variability of data was assessed through the minimum (Min) and maximum value (Max), standard deviation (SD), standard error of the mean (SE) and coefficient of variation (Cv). Given the sample sizes, the approximations of symmetry of distributions were tested by means of checking the homogeneity of data with coefficient of variation (Cv≤30%). The homogeneity of variances was tested with Levene's test for equity of variances. Testing the hypotheses on the differences in average PCC was performed with parametric analysis of variance followed by the Tukey test. The differences between the PCC registered before and the ones following the treatment within the same group of hives were tested with a dependent t-test for paired samples.

RESULTS

The results of the research are displayed with basic statistical parameters in Table 1. The average PCC in ST groups before the treatment ranged from 97.038% to 99.055%, and declined to a minimum 94.005% and maximum 97.313% following the treatment. The lowest PCC were observed in hives treated with amitraz (K+) both in a single colony – 84.020% and 90.080% - and on average in a group – 86.225% and 91.115% - at the beginning and at the end of the experiment, respectively.

In each experimental group of hives the PCC were equalized before or after the treatment (Cv<30%; Table 1). Given the values of the coefficient of variation (Table 1), the results of Levene's test (Table 2),

Table 2. Results of Levene's test and ANOVA for experimental groups evaluated before and after treatment

Time of assessment	Experimental groups	Levene's test		ANOVA	
		F	p	F	p
Before sugar treatment	ST (9 groups)	2.405	0.042*	1.825	0.116
	ST and K+ (10 groups)	2.130	0.058	50.671	<0.001**
	ST and K- (10 groups)	3.264	0.007**	1.435	0.218
	ST, K+ and K- (11 groups)	2.603	0.019*	41.456	<0.001**
After sugar treatment	ST (9 groups)	6.338	<0.001**	12.280	<0.001**
	ST and K+ (10 groups)	4.101	0.002**	24.654	<0.001**
	ST and K- (10 groups)	5.719	<0.001**	17.930	<0.001**
	ST, K+and K- (11 groups)	3.833	0.002**	30.215	<0.001**

** Very significant differences ($p \leq 0.01$)
* Significant differences ($p \leq 0.01$)

and the fact that the samples were equal in size, the testing of differences in PCC was performed by the parametric method of the analysis of variance.

The results of ANOVA (Table 2) prove the uniformity of all ST and K- groups and that the differences which appeared after the treatment resulted from the effect of sugar dust on the hygienic behavior of the bees.

The results of Tukey test (Table 3 and Fig. 1) indicate that before treatment with sugar dust, PCC in the hives treated with the acaricide (K+) was significantly lower ($p<0.001$) in comparison with all the other groups of hives (ST and K-). This points to the suppressive effect of amitraz on hygienic behavior.

Before sugar dusting, significant differences in PCC were not noticed either between the ST groups and K-, or between the ST groups (Table 3). After the application of sugar dust (Table 3 and Fig. 1), in all ST groups treated at three- and seven-day intervals the PCC was significantly ($p<0.001$) lower in comparison with K-, which indicates the suppressive effect of the treatments on hygienic behavior. Treatments at fourteen-day intervals did not result in a decrease of PCC in comparison with K-.

The results of ANOVA suggest that the PCC are very significantly influenced by the frequency of treatment with both 20 g ($F=14.583$; $p=0.002$) and 30 g of pulverized sugar ($F=42.280$; $p<0.001$), and significantly with the dose of 40 g ($F=7.506$; $p=0.012$).

The Tukey test showed that following the treatment (Table 3) with 20 g of sugar dust, the PCC was statistically very significantly lower ($p=0.004$) in the group treated at three-day intervals (ST-20-3) in comparison to the one treated at fourteen-day intervals (ST-20-14). When the dose of 30 g was applied, the PCC was very significantly lower if it was given at three- ($p<0.001$) or seven-day intervals ($p=0.001$) in comparison to application every fourteenth day. The highest sugar dust dose of 40 g produced a statistically very significant difference ($p=0.001$) in the PCC between groups treated every seventh and

Table 3. Levels of significance (p values) of differences in hygienic potential between experimental hives before and after treatment with powdered sugar (Tukey's test)

Before sugar treatment

Experimental groups	ST-20-3	ST-20-7	ST-20-14	ST-30-3	ST-30-7	ST-30-14	ST-40-3	ST-40-7	ST-40-14	K+	K-
ST-20-3		1.000	0.996	0.962	1.000	0.845	0.995	0.916	0.999	<0.001**	0.986
ST-20-7	0.290		0.941	0.998	1.000	0.977	1.000	0.993	1.000	<0.001**	1.000
ST-20-14	0.004**	0.765		0.488	0.950	0.287	0.698	0.381	0.818	<0.001**	0.605
ST-30-3	0.933	0.012*	<0.001**		0.998	1.000	1.000	1.000	1.000	<0.001**	1.000
ST-30-7	1.000	0.230	0.003**	0.963		0.972	1.000	0.991	1.000	<0.001**	1.000
ST-30-14	0.002**	0.519	1.000	<0.001**	0.001**		1.000	1.000	0.998	<0.001**	1.000
ST-40-3	0.997	0.832	0.045*	0.433	0.992	0.017*		1.000	1.000	<0.001**	1.000
ST-40-7	0.998	0.045*	<0.001**	1.000	0.999	<0.001**	0.765		1.000	<0.001**	1.000
ST-40-14	0.012*	0.933	1.000	<0.001**	0.009**	0.999	0.108	0.001**		<0.001**	1.000
K+	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**		0.000**
K-	<0.001**	0.002**	0.139	<0.001**	<0.001**	0.290	<0.001**	<0.001**	0.060	<0.001**	

After sugar treatment

Table 4. Results of testing the differences in PCCs before and after treatment

Experimental groups	Means			Dependent t-test for paired samples	
	Before treatment	After treatment	Decrease (%)	t	P
ST-20-3	98.323	94.763	-3.62	14.536	0.001**
ST-20-7	97.975	96.143	-1.87	4.398	0.022*
ST-20-14	99.055	97.105	-1.97	6.516	0.007**
ST-30-3	97.313	94.005	-3.40	10.463	0.002**
ST-30-7	98.003	94.693	-3.38	3.961	0.029*
ST-30-14	97.038	97.313	0.28	-0.357	0.745
ST-40-3	97.565	95.248	-2.37	6.278	0.008**
ST-40-7	97.175	94.285	-2.97	10.055	0.002**
ST-40-14	97.728	96.900	-0.85	1.533	0.223
K+	86.225	91.115	5.67	-5.190	0.014*
K-	97.452	98.692	1.27	-2.712	0.073

* Significant differences ($p \leq 0.05$)
** Very significant differences ($p \leq 0.01$)

every fourteenth day due to higher percentage in the former (Table 3). These results suggest that all tested doses produced a minimum decrease in the PCC when applied at fourteen-day intervals. Within each dose, no significant difference appeared if it was applied every third or seventh day (Table 3).

Comparison of the values of PCC before and after the treatment in all ST groups (Table 4) revealed that sugar in all tested doses, if applied every third day, leads to a statistically very significant decrease ($p<0.01$) in PCC. However, the effect of sugar dusting at seven-day intervals depends on the dose: doses

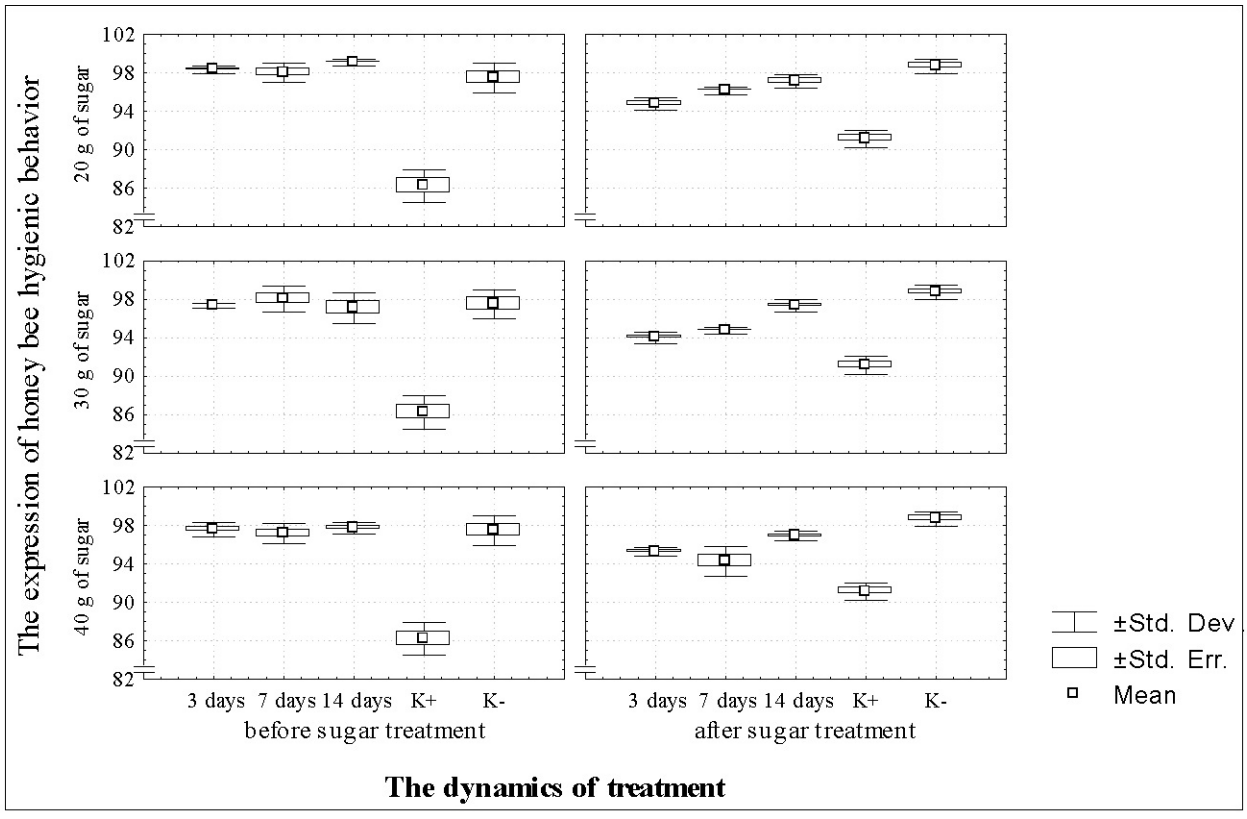

Figure 1. The expression of honey bee hygienic behavior before and after treatment with powdered sugar

of 20g and 30g resulted in a significant ($p<0.05$), and 40g a very significant ($p<0.01$) decrease. In contrast, the increase in PCC in ST-30-14 is statistically insignificant and can be considered as a consequence of the fluctuation within the sample.

DISCUSSION

In honey bees, hygienic behavior is a collective response of the adult workers to the presence of a diseased and parasitized brood (Wilson-Rich et al., 2009). Thus, knowledge of all aspects and possible influences on this behavior is of broad economic interest. Having considered our previous findings that sugar dust stimulates grooming behavior in the honey bee (Stevanovic, 2007; Cirkovic; 2011) and contributes to the fall-off of *Varroa* mites (Stanimirovic et al., 2011), the aim of the current work was to give insight into the effects of identical treatments on hygienic behavior in order to assess their safety and justifiability to be implemented in integrated pest management strategies to control *V. destructor*.

The results of the present work showed that sugar dust treatments significantly decreased the hygienic behavior, the effect being dependent on the frequency of application of each dose. More frequent treatments (every third and seventh day) with all the doses applied significantly ($p<0.001$) decreased the PCC in comparison with K-, unlike treatments at 14-day intervals. These results can be explainedable by the fact that hygienic behavior depends on both genetic and environmental factors. Hygienic behavior is a polygenic, quantitative trait (Lapidge et al., 2002; Oxley et al., 2010) which can be improved by selection; its heritability ranges between 0.18 and 0.63 (Harbo and Harris 1999; Boecking et al. 2000; Büchler et al.,

2008; Stanimirovic et al., 2008). The increase in hygienic behavior achieved by selection proved effective in improving the resistance of *A. mellifera* to *Varroa*, American and European foulbrood, and chalkbrood (Spivak and Reuter, 2001a,b; Ibrahim et al., 2007; Büchler et al., 2008). In spite of the above-mentioned major advances in knowledge of the genetic aspects of hygienic behavior, its mode of inheritance remains largely unknown (Unger and Guzman-Novoa, 2010). In addition to genetic factors, the behavioral profile of the hygienic bee is shaped by a number of other factors, including neural, social and environmental ones (Goode et al., 2006).

The treatments with sugar dust certainly act as an additional exogenous stress factor which contributes to the significant decrease in the hygienic potential. It has been established that environmental influences, both the availability of resources external to the colony (Momot and Rothenbuhler, 1971) and the state of the colony (Spivak and Gilliam, 1993; Arathi et al., 2000), can affect hygienic behavior. Colony-level expression of hygienic behavior depends on the strength of the colony (Stanimirovic et al., 2002, 2005b) and the percentage of bees capable of performing the task (Arathi and Spivak, 2001).

According to Arathi et al. (2000), the bees carrying out hygienic behavior are, on average, 15–17 days old, and contribute 18% to the population of a colony at any given moment. However, all worker bees are capable of performing hygienic tasks at some point in their adult life (Wilson-Rich et al., 2009). This is possible because age polyethism in social insect colonies is an extremely flexible system, resulting from the workers' capability to respond to changing needs determined by factors both within and outside the colony (Robinson, 1992). One of those which influence this flexibility is the necessity of a particular task (Frank and Tofts, 1994). In the current study, the decrease in the expression of hygienic behavior following sugar treatment may have resulted from a reduced need for hygienic tasks, since sugar dusting decreases the *Varroa* population (Cirkovic, 2011; Stanimirovic et al., 2011). Another possible reason may be the increased need for grooming activities (body cleaning from sugar dust) and consequential re-routing of bees from hygienic to grooming tasks. This assumption is based on our previous finding that sugar treatments significantly increase grooming behavior (Stevanovic, 2007), and is in accordance with the claims of Arathi and Spivak (2001) that the schedule of division of labor in a honeybee colony is determined more by task needs than entirely by the age demography.

More frequent treatments may have produced higher stress in the bees and thus stronger effects, possibly by alternations in the synthesis of biogenic amines, which is a common reaction to stress in insects, dependent on the intensity of the stressor and the duration of exposure (Mrdakovic et al., 2003, 2004; Peric-Mataruga et al., 2006).

It is likely that sugar dust masked the olfactory stimuli from a damaged brood and consequently led to the decrease in hygienic response, which is in accordance with the findings of Masterman et al. (2001) who claim that the detection of a parasitized brood is based on the odor which it emits, and that the hygienic behavior in bees is associated with their responses to these stimuli. This may also be important for explaining the suppressive effect of amitraz (K+) on hygienic behavior in this work. Amitraz is an agonist of octopamine, a biogenic amine in invertebrates and an analogue of adrenalin in higher animal species. Given that in *Apis mellifera* the neuromodulator octopamine, besides having other roles, is involved in olfactory learning and memory and plays a pivotal role in olfactory-based behaviors (Spivak et al. 2003; Farooqui, 2007), it is assumed that in some way amitraz influenced the perception of bees, rendering them less sensitive to olfactory stimuli. These might underlie the sharp decline in hygienic behavior in bees treated with amitraz.

Nevertheless, no sugar treatment threatened the hygienic potential, as was proven by the PPC values, which although lower, were still above 94%. Thus, it was show that these treatments are safe and can justifiably be implemented in integrated pest management strategies to control *V. destructor*.

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REFERENCES

Arathi, H.S., Burns, I. and *M. Spivak* (2000). Ethology of hygienic behavior in the the honey bee, *Apis mellifera* (Hymenoptera: Apidae): Behavioral repertoire of hygienic bees. *Ethology*, **106**, 365-379.

Arathi, H.S. and *M. Spivak* (2001). Influence of colony genotypic composition on the performance of hygienic behavior in the honey bee, *Apis mellifera* L. *Anim. Behav.* **62, 57-66.**

Bogdanov, S. (2006). Contaminants of bee products. *Apidologie*, **37**, 1–18.

Boecking, O. and *M. Spivak* (1999). Behavioral defenses of honey bees against *Varroa jacobsoni* Oud. *Apidologie*, **30**, 141-158.

Boecking, O., Bienefeld, K. and *W. Drescher* (2000). Heritability of the *Varroa*-specific hygienic behavior in honey bees (Hymenoptera: Apidae). *J. Anim. Breed. Genet*. **117**, 417-424.

Büchler, R., Garrido, C., Bienefeld, K. and *K. Ehrhardt* (2008). Selection for *Varroa* tolerance: concept and results of a long-term selection project. *Apidologie*, **39**, 598.

Cirkovic, D. (2011). The investigation on the effects of pulverised sucrose on the degree of honeybee infestation with *Varroa destructor*. PhD thesis, Belgrade University.

Cremer, S., Armitage, S. and *P. Schmid-Hempel* (2007). Social immunity. *Curr. Biol*. **17**, R693–R702.

Ellis, A.M., Hayes, G.W. and *J.D. Ellis* (2009). The efficacy of dusting honey bee colonies with powdered sugar to reduce varroa mite populations. *J. Apicult. Res*. **48,** 72-76.

Evans, J.D. and *M. Spivak* (2010). Socialized medicine: Individual and communal disease barriers in honey bees. *J. Invertebr. Pathol*. **103**, S62–S72.

Fakhimzadeh, K. (2000). Potential of super-fine ground, plain white sugar dusting as an ecological tool for the control of Varroasis in honey bee (*Apis mellifera*). *Am. Bee J*. **140**, 487-491.

Fakhimzadeh, K. (2001). The effects of powdered sugar varroa control treatments on *Apis mellifera* colony development. *J. Apicult. Res*. **40**, 105-109.

Farooqui, T. (2007). Octopamine-mediated neuromodulation of insect senses. *Neurochem. Res*. **32**, 1511-1529.

Franks, N.R. and *C. Tofts* (1994). Foraging for work: how tasks allocate workers. *Anim. Behav.* **48**, 470–472.

Goode, K., Huber, Z., Mesce, K.A. and *M. Spivak* (2006). Hygienic behavior of the honey bee (*Apis mellifera*) is independent of sucrose responsiveness and foraging ontogeny. *Horm. Behav*. **49**, 391-397.

Gregorc, A. and *I.D. Bowen* (2000). The histochemical characterisation of cell death in honeybee larvae midgut after treatment with *Paenibacillus larvae*, amitraz and oxytetracycline. *Cell Biol. Int*. **24**, 319-324.

Gregorc, A. and *J.D. Ellis* (2011). Cell death localization in situ in laboratory reared honey bee (*Apis mellifera* L.) larvae treated with pesticides. *Pestic. Biochem. Phys*. **99**, 200-207.

Harbo, J.R. and *J.W. Harris* (1999). Heritability in honey bees (Hymenoptera: Apidae) of characteristics associated with resistance to *Varroa jacobsoni* (Mesostigmata: Varroidae). *J. Econ. Entomol*. **92**, 261-265.

Ibrahim, A., Reuter, G.S. and *M. Spivak* (2007). Field trial of honey bee colonies bred for mechanisms of resistance against *Varroa destructor*. *Apidologie*, **38**, 67-76.

Lapidge, K.L., Oldroyd, B.P. and *M. Spivak* (2002). Seven suggestive quantitative trait loci influence hygienic behavior of honeybees. *Naturwissenschaften*, **89**, 565-568.

Lodesani, M., Costa, C., Serra, G., Colombo, R. and *A.G. Sabatini* (2008). Acaricide residues in beeswax after conversion to organic beekeeping method. *Apidologie*, **39**, 324–333.

Loucif-Ayad, W., Aribi, N. and *N. Soltani* (2008). Evaluation of secondary effects of some acaricides on *Apis mellifera intermissa* (Hymenoptera, Apidae): acetylcholinesterase and glutathione S-transferase activities. *Eur. J. Sci. Res*. **21**, 642-649.

Masterman, R., Ross, R., Mesce, K. and *M. Spivak* (2001). Olfactory and behavioral response thresholds to odors of diseased brood differ between hygienic and non-hygienic honey bees (*Apis mellifera* L.). *J. Comp. Physiol. A*. **187**, 441-542.

Martel, A.C., Zeggane, S., Aurieres, C., Drajnudel, P., Faucon, J.P. and *M. Aubert* (2007). Acaricide residues in honey and wax after treatment of honey bee colonies with Apivar (R) or Asuntol (R) 50. *Apidologie*, **38**, 534–544.

Milani, N. (1999). The resistance of *Varroa jacobsoni* Oud. to acaricides. *Apidologie*, **30**, 229-234.

Milani, N. and *G. Della Vedova* (2002). Decline in the proportion of mites resistant to fluvalinate in a population of *Varroa destructor* not treated with pyrethroids. *Apidologie*, **33**, 417-422.

Moritz, R.F.A., de Miranda, J., Fries, I., Le Conte, Y., Neumann P. and *R. Paxton* (2010). Research strategies to improve honeybee health in Europe. *Apidologie*, **41**, 227-242.

Momot, J.P. and *W.C. Rothenbuhler* (1971). Behavior genetics of nest cleaning in honey bees. VI: Interactions of age and genotype of bees, and nectar flow. *J. Apicult. Res*. **10**, 11-21.

Mrdaković, M., Ilijin, L., Vlahović, M., Janković-Tomanić, M., Perić-Mataruga, V., Lazarević, J. and V. *Nenadović* (2003). The effects of different constant temperatures on the activity of corpora allata in *Morimus funereus* (Coleoptera: Cerambycidae) larvae. *Arch. Biol. Sci*. **55**, 21-22.

Mrdaković, M., Ilijin, L., Vlahović, M., Janković-Tomanić, M., Perić-Mataruga, V., Lazarević J., Prolić, Z. and *V. Nenadović* (2004). The response of medial neurosecretory neurons to temperature stress in *Morimus funereus* larvae. *Arch. Biol. Sci*. **56**, 19-20.

Oxley, P.R., Spivak, M. and *B. Oldroyd* (2010). Six quantitative trait loci influence task thresholds for hygienic behavior in honeybees (*Apis mellifera*). *Mol. Ecol*. 19, 1452–1461.

Perić-Mataruga, V., Nenadović, V. and *J. Ivanović* (2006). Neurohormones in insect stress: A review. *Arch. Biol. Sci*. **58**, 1-12.

Pettis, J.S., Kochansky, J.P. and *M.F. Feldlaufer* (2004). Larval *Apis mellifera* L. (Hymenoptera, Apidae) mortality after topical application of antibiotics and dusts. *J. Econ. Entomol*. **97**, 171-176.

Robinson, G.E. (1992). Regulation of division of labor in insect societies. *Annu. Rev. Entomol.* **37,** 637–665.

Spivak, M. and *M. Gilliam* (1993). Facultative expression of hygienic behavior in honey bees in relation to disease resistance. *J. Apicult. Res*. **32**, 147-157.

Spivak, M. and *G.S. Reuter* (2001a). Resistance to American foulbrood disease by honey bee colonies *Apis mellifera* bred for hygienic behavior. *Apidologie*, **32**, 555–565.

Spivak, M. and *G.S. Reuter* (2001b). *Varroa jacobsoni* infestation in untreated honey bee (Hymenoptera: Apidae) colonies selected for hygienic behavior, *J. Econ. Entomol*. **94**, 326–31.

Spivak, M., Masterman, R., Ross, R. and *K.A. Mesce* (2003). Hygienic behavior in the honey bee (*Apis mellifera* L.) and the modulatory role of octopamine. *J. Neurobiol.* **55**, **341–354.**

Stanimirovic, Z., Pejovic, D., Stevanovic, J., Vucinic, M. and *M. Mirilovic* (2002). Investigations of hygienic behavior and disease resistance in organic beekeeping of two honeybee ecogeographic varieties from Serbia. *Acta Vet.* **52**, 169-180.

Stanimirovic, Z., Stevanovic, J., Jovanovic, S. and *M. Andjelkovic* (2005a). Evaluation of genotoxic effects of Apitol® (cymiazole hydrochloride) in vitro by measurement of sister chromatid exchange. *Mutat. Res.* **588**, 152-157.

Stanimirovic, Z., Stevanovic, J. and *D. Cirkovic* (2005b). Behavioral defenses of the honey bee ecotype from Sjenica – Pester against *Varroa destructor*. *Acta Vet*. **55**, 69-82.

Stanimirovic, Z., Stevanovic, J., Bajic, V. and *I. Radovic* (2007). Evaluation of genotoxic effects of fumagillin by cytogenetic tests *in vivo*. *Mutat. Res.* **628**, 1-10.

Stanimirovic, Z., Stevanovic, J., Mirilovic, M. and *V. Stojic* (2008). Heritability of hygienic behaviour in grey honey bees (*Apis mellifera carnica*). *Acta Vet.* **58**, 593-601.

Stanimirovic, Z., Aleksic N., Kulic, M. and *M. Maletic* (2010). Fumagillin-induced chromosome aberrations in mouse bone-marrow cells. *Arch. Biol. Sci.* **62**, 47-56.

Stevanovic, J., Stanimirovic, Z., Pejin, I.I. and *M. Lazarevic* (2006). Monitoring of mitotic index and frequency of micronuclei in evaluation of genotoxic potential of fumagillin (dicyclohexylamine) *in vivo*. *Acta Vet.* **56**, 437-448.

Stevanovic, J. (2007). Ecological-ethological defense mechanisms of *Apis mellifera carnica* against ectoparasite *Varroa destructor* on the territory of Serbia. PhD Thesis, Belgrade University.

Stevanovic, J., Stanimirovic, Z., Radakovic, M. and *V. Stojic* (2008). *In* vitro evaluation of the clastogenicity of fumagillin. *Environ. Mol. Mutagen.* **49**, 594-601.

Stanimirovic, Z., Aleksic, N., Stevanovic, J., Cirkovic, D., Mirilovic, M., Djelic, N. and *V. Stojic* (2011). The influence of pulverised sugar dusting on the degree of infestation of honey bee colonies with *Varroa destructor*. *Acta Vet.* **61,** 309-325.

Unger, P. and *E. Guzman-Novoa* (2010). Maternal effects on the hygienic behavior of Russian x Ontario hybrid honeybees (*Apis mellifera* L.). *J. Hered*. **101**, 91–96.

Wantuch, H.A. and http://www.bioone.org/doi/abs/10.1603/029.102.0603 - aff1#aff1 D.R. Tarpy (2009). Removal of Drone Brood from *Apis mellifera* (Hymenoptera: Apidae) Colonies to Control *Varroa destructor* (Acari: Varroidae) and Retain Adult Drones, *J. Econ. Entomol*. **102**, 2033-2040.

Wilson-Rich, N., Spivak, M., Fefferman, N.H. and *P.T. Starks* (2009). Genetic, individual, and group facilitation of disease resistance in insect societies. *Annu. Rev. Entomol*. **54**, 405–423.